

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460



Office of Prevention, Pesticides
and
Toxic Substances

HED DOC. NO. 013604

DATE: August 4, 1999

MEMORANDUM

SUBJECT: **PHOSMET**: Revised Report of the Hazard Identification Assessment
Review Committee on Phosmet

FROM: Linda L. Taylor, Ph.D.
Reregistration Branch I
Health Effects Division (7509C)

THRU: Jess Rowland, Chairman
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Christina Swartz
Reregistration Branch I
Health Effects Division (7509C)
and
Linda Werrell
Chemical Review Manager
Special Review and Re-registration Division 97508W)

PC Code: 059201

On July 8, 1999, the Health Effects Division's Hazard Identification Assessment Review Committee [HIARC] evaluated the recently submitted toxicology data on Phosmet and selected new toxicological endpoints for acute dietary and occupational exposure risk assessments. In a subsequent, follow-up, meeting on July 15, 1999, further discussion of the neuropathology observed in the subchronic neurotoxicity study in rats resulted in a determination that the developmental neurotoxicity study was not required for Phosmet. The HIARC previously evaluated Phosmet [HED Document No. 012449; 12/19/97], identified toxicological endpoints and dose levels of concern appropriate for use in risk assessments for different exposure routes and durations based on the available data, reassessed the reference dose [RfD] for Phosmet, and addressed the potential enhanced sensitivity of infants and children from exposure to Phosmet as required by the Food Quality Protection Act [FQPA] of 1996. **The conclusions reached at these latter meetings [7/8/99 and 7/15/99] are presented in this report. This document supersedes the previous TES and HIARC reports.**

Committee Members in Attendance

Members present were: Robert Fricke, Karen Hamernik, Susan Makris, Nancy McCarroll, Mike Ioannou, PV Shah, Virginia Dobozy, Pam Hurley, David Anderson, Jess Rowland [Co-Chairman], Brenda Tarplee [Executive Secretary].

Member *in absentia* Kathleen Raffaele, Pauline Wagner, Nicole Paquette, Tina Levine. Data were presented by Linda Taylor of Reregistration Branch I.

Also in attendance were Whang Phang, Christina Swartz, Michael Metzger, Jeff Dawson, Randy Perfetti.

Data Presentation and report Presentation:

Linda L. Taylor, Ph.D.
Toxicologist

I. BACKGROUND

On **March 3, 1994**, the Health Effects Division RfD/Peer Review Committee met to discuss and evaluate the existing toxicology data in support of Phosmet re-registration and to re-assess the Reference Dose [RfD] for this chemical in light of recently-submitted data. [**HED Document No. 012968, dated 5/11/94**].

On **March 15, 1994**, the Health Effects Division's Toxicology Endpoint Selection Committee [TES] selected doses and endpoints for Acute Dietary and Short- and Intermediate-Term Occupational and Residential risk assessments [**TES Document dated 3/15/94; HED Document No. 013496**].

On **September 4, 1997**, the Hazard Identification Assessment Review Committee [HIARC] evaluated the existing and/or recently submitted toxicology data in support of Phosmet reregistration, identified toxicological endpoints and dose levels of concern appropriate for use in risk assessments for different exposure routes and duration, and assessed/reassessed the reference dose [RfD] for Phosmet [**HIARC Report dated 12/19/97; HED Document No. 012449**].

During **May 12 through 14, 1998**, the HIARC conducted a comprehensive review of 40 organophosphates including Phosmet. At that meeting, following a consistency review of the doses and endpoints selected for dietary and non-dietary exposures, the HIARC recommended that the 10X FQPA safety factor should be reduced to 3X due to data gaps for the acute and subchronic neurotoxicity studies in rats and NTE data in the hen study [**Hazard Assessment of the Organophosphates: Report of the Hazard Assessment Review Committee dated July 7, 1998**].

On **June 15 and 16, 1998**, the FQPA Safety Factor Committee [FQPA SFC] evaluated hazard and exposure data for Phosmet and determined that the 10X to account for enhanced susceptibility of infants and children [as required by FQPA] should be reduced to 3X because of data gaps for acute and subchronic neurotoxicity studies and NTE data for the hen study [**FQPA Safety Factor Committee for the Organophosphates: A Combined Report of the Hazard Identification Assessment Review Committee and the FQPA Safety Factor Committee dated August 6, 1998**].

On **July 8, 1999**, the HIARC evaluated the recently submitted acute neurotoxicity [MRID 44673301] and subchronic neurotoxicity [MRID 44811801] studies, the new 21-day dermal toxicity study [MRID 44795801], and the new hen delayed neurotoxicity study [MRID 44587601], which previously were identified as datagaps. The HIARC determined that these studies were acceptable and, therefore, there are no data gaps for the standard Subdivision F Guidelines for Phosmet.

On **July 15, 1999**, the HIARC reassessed the significance of the neuropathology [digestion chambers in the sciatic and peroneal nerves] observed in the high-dose male rats in the subchronic neurotoxicity study. In light of the facts that these lesions occur spontaneously and are very common in all strains of rats, they occurred in males only in this study, and neuropathology was not observed in other studies on Phosmet, the HIARC determined that a developmental neurotoxicity study in rats is not required.

This report captures all of the essential conclusions reached at these meetings and supersedes the previous TES and HIARC reports.

II. HAZARD IDENTIFICATION

A. ACUTE DIETARY (Acute RfD)

Study Selected: acute neurotoxicity - rat

Guideline #: 870.6200; §81-8

MRID No.: 44673301

EXECUTIVE SUMMARY: In an acute neurotoxicity study in rats [MRID 44673301], Phosmet (94.4% purity) was administered to Sprague Dawley rats (30/sex/group) at 0, 3.0, 4.5, or 22.5 mg/kg as one dose, orally, by gavage. Body weights were recorded weekly, and clinical observations were recorded daily. Cholinesterase activity was measured in plasma, red blood cells, and brain at time of peak effect (approximately 3 hours post-dosing) on day 0, and on days 7 and 14 post-dosing, in 6 animals/sex/group. Neurobehavioral assessment (functional observation battery and motor activity testing) was performed in 12 animals/sex/group prior to the start of testing, at the time of peak effect, and on days 7 and 14 post-dosing. Brain weights were determined on days 0, 7, and 14 (nonperfused animals, 6/sex/group). At study termination, 12 animals/sex/group were perfuse in situ, and brain weight, length, and width were measured. Of the perfuse animals, 5/sex from the control and high dose groups were subjected to histopathological evaluation of brain and peripheral nervous system.

No effects of treatment were seen in the 3.0 or 4.5 mg/kg groups. At 22.5 mg/kg, there were decreases in plasma cholinesterase in both sexes, at the time of peak effect only (decreased 46% in females, 57% in males). Red blood cell cholinesterase was also decreased at the time of peak effect in both sexes (88% in females, 75% in males); inhibition persisted on days 7 (25%) and 14 (40%) for females only. Brain cholinesterase was inhibited in both sexes at all three time points (for males and females, respectively; 61 and 70% on day 0, 15 and 20% on day 7, and 9 and 17% on day 14). The only other effect of treatment was a decrease in motor activity seen in both sexes at the time of peak effect on day 0. No treatment-related changes were seen in FOB parameters or in neuropathological findings.

The NOAEL is 4.5 mg/kg, and the LOAEL was 22.5 mg/kg, based on cholinesterase inhibition [plasma, red blood cell, and brain] and decreased motor activity in both sexes.

Dose and Endpoint for Risk Assessment: NOAEL of 4.5 mg/kg; based on cholinesterase inhibition [plasma, red blood cell, and brain] and decreased motor activity in both sexes at 22.5 mg/kg.

Comments about Study/Endpoint: *This dose and endpoint replace the previous oral NOAEL based on the chronic toxicity study in rats.* The effects observed in the acute neurotoxicity study occurred after a single exposure, which is appropriate for this acute risk assessment, and the principal toxicological endpoint of concern [cholinesterase inhibition] was observed in this study.

Uncertainty Factor(UF): 100 [10 for interspecies; 10 for intraspecies]

$$\text{ACUTE RfD: } \frac{4.5 \text{ kg/kg}}{100} = 0.045 \text{ mg/kg}$$

This Risk Assessment is Required.

B. CHRONIC DIETARY [Reference Dose (RfD)]

Study Selected: chronic toxicity - rat

Guideline #: 870.4300/§83-5

MRID No.: 41916401

EXECUTIVE SUMMARY: In a 2-year chronic toxicity/carcinogenicity study [MRID 41916401], 70 control/60 treated Sprague-Dawley Crl: CD-1®(SD)BR rats/sex/group were administered Phosmet [94.3% a.i.] *via* the diet at dose levels of 0 ppm, 20 ppm [males 1.1/females 1.1 mg/kg/day], 40 ppm [males 1.8/females 2.1 mg/kg/day], 200 ppm [males 9.4/females 10.9 mg/kg/day], and 400 ppm [males 23/females 27 mg/kg/day; group terminated at 12 months] for 104 weeks.

At 20 ppm, there was marginal RBC cholinesterase (ChE) inhibition (16%) noted at 6 months in males only. At 40 ppm, RBC (about 15-20%) and serum ChE (males 5-35%; females 15-25%) was inhibited in both sexes. Brain cholinesterase was inhibited (>34%) in males and females at 200 ppm. **The LOAEL for ChE inhibition was ≤20 ppm, based on RBC ChE in males (marginal only at 6 months). The NOAEL for ChE inhibition was <20 ppm.**

Systemic toxicity was limited to an increased incidence of fatty change in the liver of males at all dose levels. In addition, at 200 ppm and above, there were increases in the incidences of depressed hepatic foci and hyperkeratosis of the stomach in males and fatty change in the liver and mineralization of the thyroid in females. At 400 ppm, body weight and body-weight gain were decreased in both sexes, and decreased kidney weight and increased BUN were observed in females. **The systemic LOAEL is ≤20 ppm, based on an increased incidence of fatty change in the liver of males. The systemic NOAEL is <20 ppm.**

Dose and Endpoint for Risk Assessment: NOAEL = 1.1 mg/kg/day, based on RBC and serum cholinesterase inhibition observed at the next higher dose level of 1.8 mg/kg/day [LOAEL].

Comments about Study/Endpoint: None.

Uncertainty Factor (UF): 100 [10 interspecies; 10 intraspecies].

$$\text{Chronic RfD: } \frac{1.1 \text{ mg/kg/day}}{100} = 0.011 \text{ mg/kg/day}$$

This Risk Assessment is Required.

C. OCCUPATIONAL / RESIDENTIAL EXPOSURE

1. DERMAL ABSORPTION

Study Selected: dermal absorption

Guideline #: §85-2; 870.7600

MRID No.: 40122201

Executive Summary: In a dermal absorption study [MRID 40122201], Phosmet [Imidan 50-WP, 50%] was applied to the shaved back of 4 male Sprague-Dawley (CD) rats/dose. Dilutions used were 1:2, 1:10, and 1:100 applied at a rate of 300 µL/rat. Administered doses were 2.67, 0.52, and 0.058 mg/cm² skin. The dosing solutions contained 20-50 µCi of labeled compound. The radioactive test

material had a specific activity of 26.6 mCi/mmol and was 97% pure. Phosmet was poorly absorbed when applied to the shaved skin of rats. The percent of radioactive dose found in the carcass, skin, urine, feces, and blood (combined) after 24 hours was 0.9, 3.8, and 11.8% of the administered doses of 2.67, 0.52 and 0.058 mg/cm² skin, respectively. The skin at the dosing site contained much of the radioactivity. The amount in the carcass and excreta reached a maximum at 24 hours and accounted for 7.9, 1.7, and 0.3% of the administered radioactivity at the low-, mid-, and high-doses, respectively. Excretion of the absorbed radioactivity was primarily urinary; 0.1% of the high-dose (1:2 dilution), 1.1% of the mid-dose (1:10 dilution), and 5.4% of the low-dose (1:100 dilution) radiolabel was found in the urine between 10 and 24 hours. Much lower amounts were found in the feces.

Dermal Absorption Factor for Use in Risk Assessment: 10%. This factor is required only for Intermediate-Term Dermal Exposure of greater than 30-days duration since a dermal NOAEL is used for the Short-Term and Intermediate-Term Dermal Exposures of less than 30-days duration.

2. SHORT-TERM DERMAL (1 - 7 days)

Study Selected: 21-day dermal toxicity study - rat

Guideline #: §82-2/870.3200

MRID No.: 44795801

EXECUTIVE SUMMARY: In a subchronic dermal toxicity study (MRID 44795801) Imidan 70-WSB (71.2% a.i., Lot #06207139) was dermally applied to the backs of 10 Sprague-Dawley (CrI:CD VAF/Plus) rats/sex/dose in deionized distilled water at dose levels of 0, 15, 22.5 or 60 mg/kg/day for 6 hours/day, 5 days/week, for 3 weeks.

No deaths occurred. There were no treatment-related effects on body weight, food consumption, clinical signs of toxicity, hematology, organ weights, or macroscopic and microscopic examination of the tissues. Plasma ChE was significantly decreased in males in the 22.5 and 60 mg/kg/day groups (33 %) and in females in the 60 mg/kg/day group (38 %). RBC ChE was significantly decreased in females in the 60 mg/kg/day group (29 %). Brain ChE was significantly decreased in males in the 60 mg/kg/day group (30 %) and in females in the 22.5 and 60 mg/kg/day groups (20 and 66 %, respectively). **The LOAEL is 22.5 mg/kg/day, based on decreases in plasma ChE in males and brain ChE in females. The NOAEL is 15 mg/kg/day .**

Dose and Endpoint for Risk Assessment: NOAEL of 15 mg/kg/day; based on decreases in plasma ChE in males and brain ChE in females at the LOAEL of 22.5 mg/kg/day.

Comments about Study/Endpoint: *This dose and endpoint replace the previous oral NOAEL based on the chronic [oral] toxicity study in rats.* The 21-day dermal study was performed using the more appropriate route of exposure [**dermal**] for this assessment, and the endpoint [cholinesterase inhibition] is appropriate.

This Risk Assessment is Required.

3. INTERMEDIATE-TERM DERMAL (1-Week to Several Months)

A. FOR EXPOSURES <30 days

Study Selected: 21-day dermal toxicity - rat

Guideline #: §82-2/870.3200

MRID No.: 44795801

Executive Summary: see under Short-Term

Dose and Endpoint for Risk Assessment: NOAEL of 15 mg/kg/day; based on decreases in plasma ChE in males and brain ChE in females at the LOAEL of 22.5 mg/kg/day.

Comments about Study/Endpoint: This study and dose are appropriate for the majority of exposure scenarios, which are thought to be of less than 30 days. In the event that exposures of greater than 30 days are identified, the HIARC selected the subchronic neurotoxicity study [see below under B] as appropriate for such exposure scenarios.

This Risk Assessment is Required.

B. FOR EXPOSURES >30 days

Study Selected: subchronic neurotoxicity - rat

Guideline #: §82-7/870.6200

MRID No.: 44811801

EXECUTIVE SUMMARY: In a subchronic oral neurotoxicity study [MRID 44811801], Phosmet [97.3% a.i.] was administered for 90 days to 32 CrI:CD(SD)IGS BR rats/sex/dose at nominal dietary concentrations of 0, 25, 50, and 150 ppm. **Actual mean dietary concentrations of Phosmet based on analysis of weekly batches of diet were 0, 21.6, 39.7, and 136 ppm (equivalent to achieved doses of 0/0, 1.5/1.6, 2.7/3.1, and 9.4/11.0 mg/kg/day [M/F]).** Cholinesterase activity levels were determined using modified Ellman method in the blood and brains of 2 rats/sex/dose during the pretest and 6 rats/sex/dose during study weeks 3, 7, and 13. The remaining 12 rats/sex/dose were subjected to the functional observational battery (FOB) and motor activity measurements during the pretest period and study weeks 3, 7, and 12 and were then perfuse *in situ* at study termination. Five rats/sex from the control and high-dose groups were used for neuropathological examination.

No rats died during the study. No changes in appearance or behavior, body weights or body-weight gains, or food consumption were observed in any of the treated groups when compared to concurrent controls. Results of the FOB indicated no treatment-related findings during the home cage, handling, open field, sensory, neuromuscular, physiological, and locomotor activity observations. There were no adverse effects on mean ambulatory, total motor activity, or gross neurological findings. No treatment-related effects were observed on brain weight in non-perfuse and perfuse animals. An increase in the incidence of neuropathological changes [characterized by digestion chambers in the sciatic and peroneal nerves] was observed in the high-dose perfuse males.

In general, whole blood and red blood cell (RBC) cholinesterase activity levels were decreased in all dose groups of both sexes. Plasma and regional brain cholinesterase activities were

decreased in all dose groups of the treated females. The details are summarized below:

In the 22 ppm group, mean RBC cholinesterase activity was significantly decreased ($p < 0.01$) in the males at week 13 (19%) and females at week 7 (42%). Significant decreases ($p < 0.05$ or 0.01) were also observed at week 13 in mean plasma cholinesterase activity in the females (29%), mean whole blood cholinesterase in both sexes (16%-19%), and cholinesterase activity of the olfactory bulb (36%) and brain stem (21%) regions in the females.

In the 40 ppm group, the following significantly decreased ($p < 0.05$ or 0.01) mean cholinesterase activity levels were observed: (i) plasma cholinesterase in the males at week 3 (21%) and females at weeks 3 and 13 (27%-46%); (ii) RBC cholinesterase in the males at weeks 3, 7, and 13 (26%-39%) and females at week 7 (38%); (iii) whole blood cholinesterase at weeks 3, 7, and 13 in both sexes (24%-36%); (iv) whole blood cholinesterase at week 7 in the females (20%) and at weeks 3 and 7 in the males (11%-17%).

In the 136 ppm group, the following decreased ($p < 0.05$ or 0.01) mean cholinesterase activity levels were observed: (i) plasma cholinesterase at weeks 3, 7, and 13 in both sexes (23%-71%); (ii) RBC cholinesterase at weeks 3, 7, and 13 in males (65%-70%) and at weeks 3 and 7 in females (66%-89%); (iii) whole blood cholinesterase in both sexes (59%-74%) at weeks 3, 7, and 13; (iv) whole brain cholinesterase in both sexes (43%-68%) at weeks 3 and 7; and (v) in all six brain regions in the females (36%-67% and in all brain regions except the olfactory region in the males (27%-52%) at week 13.

The LOAEL is 22 ppm (equivalent to 1.5/1.6 mg/kg/day [Male/Female], the LDT), based on dose-related decreases in plasma, RBC, whole blood, and brain cholinesterase activity levels. The NOAEL was not established.

Dose and Endpoint for Risk assessment: LOAEL is 1.5 mg/kg/day; based on dose-related decreases in plasma, RBC, whole blood, and brain cholinesterase activity; a NOAEL was not established.

Comments about Study/Endpoint: In the event that exposures of greater than 30 days are identified, the HIARC identified this study and endpoint. *This dose and endpoint replace the previous oral NOAEL based on the chronic [oral] toxicity study in rats.* The endpoint [cholinesterase inhibition] is appropriate, and the duration of the study is appropriate for an exposure period of greater than 30 days]. Since this is an **oral** study, route-to-route extrapolation should be performed for this risk assessment. The dermal absorption factor is 10%.

4. LONG-TERM DERMAL (Several Months to Lifetime)

This Risk Assessment Is Not Required, based on use pattern.

5. SHORT-TERM INHALATION (1 - 7 days)

Study Selected: acute neurotoxicity - rat

Guideline #: §81-8; OPPTS 870.6200

MRID No.: 41916401

EXECUTIVE SUMMARY: see under Acute Dietary

Dose and Endpoint for Risk Assessment: NOAEL = 4.5 mg/kg, based on cholinesterase inhibition [plasma, red blood cell, and brain] and decreased motor activity observed at the next dose level of 22.5 mg/kg.

Comments about Study/Endpoint: *This dose and endpoint replace the previous oral NOAEL based on the chronic [oral] toxicity study in rats.* The endpoint [ChEI] is appropriate and the effects occurred following an acute exposure. Since this is an **oral** study, appropriate route-to-route extrapolation should be performed for this risk assessment.

This Risk Assessment Is Required.

6. INTERMEDIATE-TERM INHALATION (1-Week to Several Months)

Study Selected: subchronic neurotoxicity - rat

Guideline #: §82-7; OPPTS 870.6200

MRID No.: 44811801

EXECUTIVE SUMMARY: see under Intermediate-Term Dermal [>30 days exposure]

Dose and Endpoint for Risk Assessment: For exposures < 30 days: 1.5 mg/kg/day is NOAEL at 3-week interval in this study for cholinesterase inhibition [plasma, RBC, whole blood, and brain]. For exposures > 30 days: 1.5 mg/kg/day is LOAEL at the 3-month interval for cholinesterase inhibition.

Comments about Study/Endpoint: *This dose and endpoint replace the previous oral NOAEL based on the chronic [oral] toxicity study in rats.* The endpoint [ChEI] is appropriate and the effects occurred following repeat exposure up to 90 days. Since this is an **oral** study, appropriate route-to-route extrapolation should be performed for this risk assessment.

This Risk Assessment Is Required.

Only an acute inhalation toxicity study is available in the data base. Therefore, the HIARC selected oral values for inhalation exposure risk assessments. The inhalation risk assessments should follow the route-to-route extrapolation, as outlined below.

7. LONG-TERM INHALATION (Several Months to Lifetime)

This Risk Assessment Is Not Required based on use pattern.

D. MARGIN OF EXPOSURE

A margin of exposure [MOE] of 100 is adequate for short-term dermal and inhalation occupational exposures and intermediate-term dermal and inhalation occupational exposures of less than 30 days. However, if it is determined that exposures of greater than 30 days occur, a MOE of 300 is required for intermediate-term dermal and inhalation exposures of due to the use of a LOAEL. The MOE for residential exposure will be determined by the FQPA Safety Factor Committee [see FQPA Safety Factor Committee Report, HED Document No. 013584, dated 7/21/99].

E. RECOMMENDATION FOR AGGREGATE EXPOSURE RISK ASSESSMENTS

For both **short- and intermediate-term aggregate exposure risk assessments**, the Aggregate Risk Index [ARI] should be used since different MOEs are required for short term [MOE=100] and intermediate term [MOE=300] dermal and inhalation exposure risks. However, it should be noted that the aggregate systemic [oral], dermal, and inhalation exposure risk assessments **are appropriate** due to the common toxicological endpoint [cholinesterase inhibition] observed *via* the three routes of exposure.

III. CLASSIFICATION OF CARCINOGENIC POTENTIAL

The HED Carcinogenicity Peer Review Committee [Document No. 010998] classified Phosmet as a Group C - possible human carcinogen and recommended that for the purpose of risk characterization, the Reference Dose [RfD] approach should be used for quantitation of human risk [memo dated 5/26/94]. This decision was based on an increased incidence of liver tumors in male B6C3F1 mice at the high dose, that was statistically significant by pair-wise comparison, with a statistically significant trend and which also had an apparent early onset. Female mice had a significant dose-related trend for liver tumors and for mammary gland adenocarcinomas as well. There was no evidence for carcinogenicity in an acceptable study in rats. Phosmet was determined by the CPRC to be a potent direct-acting mutagen. NOTE: Since the CPRC assessment, the Agency has developed new guidelines for risk assessment with respect to carcinogenesis, and it was determined that a Q_1^* should be generated for Phosmet for use in risk assessment. The Q_1^* for Phosmet is $3.58 \text{ (mg/kg/day)}^{-1}$, based on male mouse liver tumors combined.

1. Combined Chronic Toxicity/Carcinogenicity Study in Rats

Executive Summary: see under Chronic Dietary

MRID No. MRID 41916401

Discussion of Tumor Data Discussion of Tumor Data There were no significant compound-related tumors observed in either sex.

Adequacy of the Dose Levels Tested The 200 ppm dose was considered adequate for assessing the carcinogenic potential of Phosmet, based on decreased brain cholinesterase activity in both sexes. Adequacy of the dosing is supported by the non-neoplastic liver effects at doses 200 ppm.

2. Carcinogenicity Study in Mice

Executive Summary: Groups of B6C3F1 mice (50/sex/group) were fed diets containing Phosmet at doses of 0, 5, 25, and 100 ppm [males 1.0, 4, 14 mg/kg/day/females 1.2, 5, 18 mg/kg/day] for 2 years. An additional 10 mice/sex/group were treated and sacrificed at week 52. Survivors were sacrificed at the termination of the study.

MRID No. Accession No. 254608, 245609

Discussion of Tumor Data: Male mice had increases in hepatocellular adenomas and combined adenoma/carcinoma that were statistically significant by pair-wise comparison of the HDT with controls, and a statistically-significant increasing trend. Female mice had a significantly increasing trend in carcinoma and combined adenoma/carcinoma. At the interim sacrifice, there was an increasing trend in the incidence of combined hepatocellular adenoma/carcinoma in male mice. Females did not have liver tumors at this time. Female mice also had a statistically-significant increasing trend in the incidence of mammary gland adenocarcinomas. This tumor was considered to be uncommon.

Adequacy of the Dose Levels Tested: Dosing was considered adequate for carcinogenicity testing, based on decreased plasma and brain cholinesterase activity in both sexes, testicular changes in males, and liver changes in both sexes at the high-dose level.

3. Mutagenicity Data

MRIDs: 00164884, 00164885, 00164886, 00164887, 00164888, 40199401

It was concluded that Phosmet is a **very potent, direct-acting, mutagen**.

Phosmet was negative when tested in a reversion assay using *Escherichia coli* strains B/r WO2 hcr+ and WP2 hcr- and in a rec-assay with *Bacillus subtilis* strains H17 Rec+ and M45 Rec - without activation. A positive response was obtained in an Ames test using *Salmonella typhimurium* [TA 100, TA98, TA1535, TA1537, TA1538] only in strain TA 100 without activation. All other strains tested, with and without activation, were negative. A dominant lethal test in the rabbit proved inconclusive. In a *Salmonella typhimurium* Reverse Mutation assay [TA 100, TA 1535], Phosmet was positive with and without metabolic activation. Phosmet was also positive with and without activation in a mouse lymphoma forward mutation assay, positive for structural chromosomal aberrations without activation, positive for SCE with and without activation, and positive in the morphological transformation of BALB.3T3 cells. The DNA damage assay in human fibroblasts was negative with and without activation.

IV. FQPA CONSIDERATIONS

1. Adequacy of the Data Base

All required guideline studies are available. There are adequate rat and rabbit developmental toxicity studies on Phosmet. An adequate acute neurotoxicity study in rats and an adequate subchronic

neurotoxicity study in rats are available on Phosmet. There is an adequate 2-generation reproduction study in rats on Phosmet.

2. Neurotoxicity Data

A. In an acute delayed neurotoxicity study [MRID 44587601], a group of 24 adult female domestic hens were given a single oral dose of Phosmet [97.4%] in corn oil at a dose of 600 mg/kg by oral gavage and observed for 21/22 days. The dose of Phosmet employed in this study was greater than the oral LD₅₀ [577 mg/kg]. Groups of 12 female hens were given either the vehicle control (corn Oil) or the positive control (TOCP) at 1000 mg/kg. To protect the Phosmet-treated birds from acute toxicity, atropine [at 20 mg/kg] was given by subcutaneous injection prior to dosing.

All Phosmet treated hens showed signs of unsteadiness, subdued behavior, recumbency, and salivation from days 1-8. In the neurotoxicity section of the report, hens dosed at 600 mg/kg were subdued, unsteady, and recumbent from days 1-5. Treatment-related decreases in body weight of 8%-15% were seen in the Phosmet treated hens during the first week of dosing. Body weights recovered by day 21. No ataxia or decreases in brain and spinal cord NTE were observed in Phosmet treated hens. Brain acetylcholinesterase was decreased 63% in hens treated with Phosmet. No increase in the pathology of the peripheral nerve, spinal cord, or brain was seen in Phosmet treated hens. TOCP treated hens showed a decrease in brain and spinal cord NTE, an increase in ataxia, and lesions in the brain, spinal cord, and peripheral nerve.

B. In an acute neurotoxicity study in rats [MRID 44673301], Phosmet (94.4% purity) was administered to Sprague Dawley rats (30/sex/group) at 0, 3.0, 4.5, or 22.5 mg/kg as one dose, orally, by gavage. [See **EXECUTIVE SUMMARY under Acute Dietary**].

The NOAEL is 4.5 mg/kg, and the LOAEL was 22.5 mg/kg, based on cholinesterase inhibition [plasma, red blood cell, and brain] and decreased motor activity in both sexes.

C. In a subchronic oral neurotoxicity study [MRID 44811801], Phosmet [97.3% a.i.] was administered for 90 days to 32 CrI:CD(SD)IGS BR rats/sex/dose at nominal dietary concentrations of 0, 25, 50, and 150 ppm. See **EXECUTIVE SUMMARY under Intermediate-Term Dermal**.

In general, whole blood and red blood cell (RBC) cholinesterase activity levels were decreased in all dose groups of both sexes. Plasma and regional brain cholinesterase activities were decreased in all dose groups of the treated females.

The LOAEL is 22 ppm (equivalent to 1.5/1.6 mg/kg/day [Male/Female], the LDT), based on dose-related decreases in plasma, RBC, whole blood, and brain cholinesterase activity levels. The NOAEL was not established.

An increase in the incidence of neuropathological changes [characterized by digestion chambers in the sciatic and peroneal nerves] was observed in the high-dose perfuse males. In discussions with an expert on neuropathology [Dr. David Eisenbrandt], it was determined that these are spontaneous lesions, which occurred in males only, and were classified a “minimal” by the testing facility’s pathologist. Based on this, and the fact that neuropathological lesions were not observed in any other study on Phosmet, the HIARC concluded that a developmental neurotoxicity study in rats would not be required for Phosmet. As confirmatory data, characterization of the lesion with respect to the

definition of “minimal” severity is required; i.e., data on how many nerve fibers were affected in each case compared to the same information for the historical control incidence. Additionally, the actual incidence observed in each of the historical control studies is required as confirmatory data.

EVIDENCE OF NEUROTOXICITY FROM OTHER ORAL TOXICITY STUDIES

In a **rat developmental toxicity study** [MRID 41962902], treatment-related clinical signs were observed at the 15 mg/kg/day dose level, which consisted of tremors/shaking and subdued behavior.

In the **mouse carcinogenicity study** [MRID 42642401], convulsions were observed in males at 25 ppm.

In a **2-generation reproduction study in rats** [MRID 41520001], tremors were observed at the high-dose level [23.4 mg/kg/day].

In the **developmental toxicity study in rabbits** [MRID 41962901], unsteady gait and shaking were observed at 15 mg/kg/day.

3. Developmental & Reproductive Toxicity

No evidence of enhanced susceptibility was observed in the developmental toxicity studies in rats and rabbits or in a two-generation reproduction study in rats. The details and previous assessment by the HIARC [May 12-14, 1998] are summarized below.

A. Prenatal Developmental Toxicity Study in Rats

The NOAEL, LOAEL and endpoint selected for maternal and developmental toxicity in the prenatal developmental toxicity study in rats are shown in Table 1. **No evidence of enhanced susceptibility** was observed for Phosmet following *in utero* exposure to pregnant rats. There was no evidence of effects being produced in fetuses at lower doses as compared to maternal rats nor was there evidence of an increase in severity of effects at or below maternally toxic doses.

Table 1. NOAELs/LOAELs & ENDPOINTS FOR DEVELOPMENTAL TOXICITY STUDIES IN RATS						
CHEMICAL	MATERNAL TOXICITY			DEVELOPMENTAL TOXICITY		
	(mg/kg/day)		ENDPOINT	(mg/kg/day)		ENDPOINT
	NOAEL	LOAEL		NOAEL	LOAEL	
PHOSMET	10	15	Decreased body weight gain and food consumption and clinical signs.	≥15	NA	No developmental toxicity at HDT.

B. Prenatal Developmental Toxicity Study in Rabbits

For Phosmet, the NOAEL and LOAEL were the **same for maternal and developmental toxicity** (i.e., fetal effects were seen at the same dose that caused maternal toxicity) **but the developmental (fetal) effects** appeared to be **more severe** [Table 2].

Table 2. NOAELs/LOAELs & ENDPOINTS FOR DEVELOPMENTAL TOXICITY STUDIES IN RABBITS						
CHEMICAL	MATERNAL TOXICITY			DEVELOPMENTAL TOXICITY		
	(mg/kg/day)		ENDPOINT	(mg/kg/day)		ENDPOINT
	NOAEL	LOAEL		NOAEL	LOAEL	
PHOSMET	5	15	Clinical signs and decreased body weight.	5	15	Increased incidence of skeletal variations in fetuses.

Following a qualitative evaluation of the effects observed, the HIARC concluded that fetal effects occurred at dose levels causing similar or more severe maternal toxicity. The rationale for this conclusion is provided in Table 3.

Table 3. DEVELOPMENTAL TOXICITY SEEN IN THE PRESENCE OF MATERNAL TOXICITY	
PHOSMET	The dose that induced clinical signs [unsteady gait, shaking, salivation, and irregular breathing] and decreased body weight in dams also resulted in skeletal variations in the fetuses.

C. Two-Generation Reproduction Study in Rats

The NOAEL, LOAEL, and endpoints selected for the parental systemic and offspring toxicity in the two-generation reproduction study is shown in Table 4. **No evidence of enhanced susceptibility** was observed for Phosmet following pre and/or postnatal exposure in the two-generation reproduction study in rats [i.e., effects noted in offspring occurred at maternally toxic doses or higher].

Table 4. NOAELs/LOAELs & ENDPOINTS FOR THE 2-GENERATION REPRODUCTION TOXICITY STUDIES IN RATS						
CHEMICAL	PARENTAL SYSTEMIC TOXICITY			OFF SPRING TOXICITY (mg/kg/day)		
	(mg/kg/day)		ENDPOINT	(mg/kg/day)		ENDPOINT
	NOAEL	LOAEL		NOAEL	LOAEL	
PHOSMET	≤1.5	6.1	RBC ChEI.	1.5	6.1	Decreased number of live pup/litter, pup weights, fertility index and lactation index.

4. Determination of Susceptibility

At the May 12-14, 1998 meeting, the HIARC concluded that there is **no evidence of increased sensitivity**, based on an adequate 2-generation reproduction study in rats, an adequate rat and an adequate rabbit developmental toxicity study. There is **evidence of neurotoxicity** in rats and rabbits [clinical signs: tremors, shaking, decreased activity, unsteady gait, subdued mood convulsions].

5. Determination of the Need for Developmental Neurotoxicity Study

At the May 12-14, 1998 meeting, the HIARC placed the requirement for a developmental neurotoxicity study in reserve status, pending the receipt and evaluation of the acute and subchronic neurotoxicity studies in rats. Both of these studies have been submitted and evaluated.

- (I) Evidence supporting a developmental neurotoxicity study

Phosmet is a neurotoxic organophosphate. There is evidence of neurotoxicity [tremors, subdued behavior, shaking, unsteady gait, convulsions] in studies in rats [developmental toxicity and 2-generation reproduction], rabbits [developmental toxicity], and mice [carcinogenicity]. Brain cholinesterase inhibition has been observed in every study in which it has been measured and in every species tested [rat, mouse, and dog].

(ii) Evidence that does not support a developmental neurotoxicity study

No increased susceptibility in rats or rabbits following *in utero* exposure or in rats following pre- and/or post natal exposure. There is no evidence of developmental toxicity, other than skeletal variations observed in the rabbit. Neuropathology was not observed in any of the seven studies performed on Phosmet in which the brain, nerves, and spinal cord were examined microscopically.

The HIARC concluded that a developmental neurotoxicity study was not required based on the currently-available data.

4. Recommendation for the FQPA Factor

Previously, the HIARC [HED Document No. 012449, dated 12/19/97] and FQPA Safety Factor Committee [combined report dated 8/6/98] recommended a 3X uncertainty factor/FQPA safety factor for FQPA considerations due to data gaps.

On July 8, 1999, the HIARC reviewed the recently submitted acute and subchronic neurotoxicity studies with Phosmet in rats and the NTE data for the hen study, which were previously identified as data gaps. The HIARC determined that these studies were acceptable and therefore, there were no data gaps for the standard Subdivision F Guideline requirements for a food-use chemical by 40 CFR Part 158.

At this meeting, however, the HIARC determined that a developmental neurotoxicity study in rats is required due to the evidence of neuropathology in the subchronic neurotoxicity study in rats that cannot be discounted. The neuropathology observed in this study was described as minimal digestion chambers in sciatic nerves of 3 of 5 male rats at the high dose. The Committee members were not sure of the significance of these lesions at the time of the HIARC meeting, and in the interest of time as well as to be protective, the HIARC recommended that a DNT be required.

On July 15, 1999, Phosmet was brought back to the HIARC to re-evaluate the neuropathology observed in the subchronic neurotoxicity study in rats. David Eisenbrandt, pathologist with Global Toxicology Dow AgroSciences joined the Committee *via* telephone to characterize the digestion chambers observed in the sciatic nerve fibers in the study. In summary, these lesions occur spontaneously and are very common in all strains of rats. Based on the information provided and the fact that neuropathology was not observed in other studies on Phosmet, **the HIARC revoked the requirement for a developmental neurotoxicity study in rats.**

Since all data requirements have been satisfied, the HIARC recommended that the FQPA safety factor be removed [1X]. See FQPA Safety Factor Committee Report dated July 21, 1999, HED Document No. 013584].

V. DATA GAPS

There are no data gaps *per se*. However, **confirmatory data** are required. For the 21-day dermal toxicity study [MRID 44795801], in order to verify the NOAEL, historical control data for cholinesterase activity [plasma and brain] are required, as well as a statistical analysis of the combined control cholinesterase data [concurrent and “expanded” controls generated for the study] with the Phosmet cholinesterase data. For the subchronic neurotoxicity study in rats [MRID 44811801], characterization of the lesion [digestion chamber] in the sciatic/peroneal nerves with respect to the definition of “minimal” severity is required; i.e., data on the number of digestion chambers per nerve; how many fibers were affected in each case compared to the same information for the historical control incidence. Additionally, the actual incidence observed in each of the historical control studies [# of rats with lesion/# of rats examined] should be submitted.

VI. HAZARD CHARACTERIZATION

Phosmet [O,O-dimethyl phosphorodithioate S-ester with N-(mercaptomethyl)phthalamide] is an organophosphate insecticide registered for use against a wide range of insects such as alfalfa weevil, boll weevil, codling moth, grape berry moth, leafrollers, Oriental fruit moth, plum curculio, twig borers, and others.

Phosmet is a cholinesterase inhibitor, and it produces the associated clinical signs, such as tremors, shaking, unsteady gait, subdued mood, decreased activity, salivation, muscle weakness, convulsions in rats and rabbits [2-generation reproduction (rat) and developmental toxicity studies (rats and rabbit)] and decreased cholinesterase activity [plasma, RBC, brain] in rats, mice, and dogs following acute, subchronic and chronic exposures.

In acute toxicity studies, Phosmet exhibits severe toxicity *via* the oral and inhalation routes of exposure. Phosmet is not acutely toxic in rats *via* the dermal route, is non-irritating to the skin, and is not an eye irritant in the rabbit.

Phosmet did not cause acute delayed neurotoxicity in hens, and there was no evidence of neuropathology in the acute and chronic studies in rats, in the chronic dog study, or the mouse long-term study. No treatment-related effects were observed on motor activity or in the functional observation battery parameters measured in the acute and subchronic neurotoxicity studies in rats.

Phosmet did not produce developmental or reproductive toxicity, and there is no indication of an increased sensitivity of offspring in rats or rabbits after prenatal and/or postnatal exposure to Phosmet.

Phosmet produced increases in the incidence of hepatocellular adenomas and combined adenoma/ carcinoma in male B6C3F1 mice, which were statistically significant by pair-wise comparison of the high-dose with controls and there was a statistically-significant trend. In female mice, there was a statistically-significant increasing trend in carcinomas and combined adenoma/carcinoma. Additionally, at the interim sacrifice, there was a statistically-significant increasing trend in the incidence of combined hepatocellular adenoma/carcinoma in male mice. Female mice also had a statistically-significant increasing trend in the incidence of mammary gland adenocarcinomas. This tumor was considered uncommon. No increased incidence in any tumor was observed in Sprague-Dawley rats. Phosmet is a potent, direct-acting mutagen,

which tested positive in several of the required mutagenicity battery assays. The HED Cancer Peer Review Committee classified Phosmet as a Group C - possible human carcinogen and recommended that for the purposes of risk characterization, the Reference Dose [RfD] approach should be used for quantitation of human risk. **Based on the current Agency policy with respect to quantification of risk, a Q_1^* was calculated for Phosmet and it should be used for quantification of human cancer risk.**

CHOLINESTERASE INHIBITION

MOUSE Cholinesterase inhibition [brain and plasma] was observed in mice of both sexes in the carcinogenicity study at the 12-month and terminal sacrifices, and brain cholinesterase inhibition was significant at all dose levels in the females at study termination [1 mg/kg/day LDT]. There was an increased incidence of convulsions in the treated male groups associated with handling during the second year of the study. **RAT** In the chronic rat study, RBC cholinesterase was significantly decreased in both sexes at the mid- [6, 18, and 24 months] and high-dose [6, 12, 18, and 24 months] levels, and brain cholinesterase inhibition was observed at the high-dose level in both sexes, but no clinical signs were observed. At a higher dose level that was administered for 12 months only, decreased plasma, RBC, and brain cholinesterase activities were observed at the 6- and 12-month intervals in both sexes. In the 2-generation reproduction study, there were significant, dose-related, decreases in RBC cholinesterase activities in the F0 and F1 females and in the F0 males at study termination. Serum cholinesterase activity was inhibited in both sexes and both generations at the high-dose level, and F0 females and both sexes of the F1 generation displayed significant serum cholinesterase activity inhibition also. Clinical signs [tremors (F0 & F1); convulsion, decreased activity, muscle weakness (F1)] were observed in several females at the high dose only. Clinical signs [tremors, shaking, subdued mood, unsteady gait, salivation] were observed in female rats and rabbits exposed *via* gavage in the developmental toxicity studies; cholinesterase measurements are not obtained in this type of study. In both the acute and subchronic neurotoxicity studies, cholinesterase activity was inhibited to a high extent in the absence of clinical signs of cholinesterase inhibition.

VI. ACUTE TOXICITY

Acute Toxicity of Phosmet

Guideline No.	Study Type	MRIDs #	Results	Toxicity Category
81-1	Acute Oral - rat	00046189	LD ₅₀ = 113 mg/kg	II
81-2	Acute Dermal - rabbit	00046190	LD ₅₀ >5000 mg/kg	III
81-3	Acute Inhalation - rat	00063197	LC ₅₀ >0.152 mg/L	I
81-4	Primary Eye Irritation	00046192	moderate eye irritant	III
81-5	Primary Skin Irritation	00046191	not a skin irritant	IV
81-6	Dermal Sensitization	no study		N/A
81-7	Delayed Neurotoxicity	44587601	unsteadiness, subdued behavior, recumbency, salivation; no ataxia; no decreases in brain or spinal cord NTE; brain ChE decreased 63%; no neuropathology.	N/A
81-8	Acute Neurotoxicity	44673301	NOAEL 4.5 mg/kg LOAEL 22.5 mg/kg, based on cholinesterase inhibition [plasma, RBC, brain] and decreased motor activity in both sexes.	N/A

VII. SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

The doses and toxicological endpoints selected for various exposure scenarios are summarized below.

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary	NOAEL 4.5	Cholinesterase inhibition [plasma, RBC, brain] and decreased motor activity	Rat Acute Neurotoxicity
Chronic Dietary non-carcinogenic effects	NOAEL=1.1 (UF=100)	Decreased RBC and serum cholinesterase	Rat Chronic Toxicity/Carcinogenicity
		Chronic RfD = 0.011 mg/kg/day	
Chronic Dietary carcinogenic effects	$Q_1^* = 3.58$ (mg/kg/day) ⁻¹	male mouse liver tumors combined	
Short-Term (Dermal)	dermal NOAEL = 15	brain (females)/plasma (males) cholinesterase inhibition	Rat 21-day dermal toxicity
Intermediate-Term (Dermal <30 days)	dermal NOAEL = 15	brain (females)/plasma (males) cholinesterase inhibition	Rat 21-day dermal toxicity
Intermediate-Term* (Dermal >30 days)	oral LOAEL = 1.5	brain, plasma, whole blood, RBC cholinesterase inhibition	Rat subchronic neurotoxicity
Short-Term (Inhalation)*	oral LOAEL 4.5	Cholinesterase inhibition [plasma, RBC, brain] and decreased motor activity	Rat Acute Neurotoxicity
Intermediate-Term (Inhalation <30 days)*	oral NOAEL = 1.5	brain (females)/plasma (males) cholinesterase inhibition	Rat subchronic neurotoxicity
Intermediate-Term (Inhalation >30 days)*	oral LOAEL = 1.5	brain (females)/plasma (males) cholinesterase inhibition	Rat subchronic neurotoxicity
Long-Term (Dermal & Inhalation)	Is not required due to minimal concern for exposure <i>via</i> these routes of exposure, based on the use pattern.		

* appropriate route-to-route extrapolation should be performed for these risk assessments. Exposure values using a dermal absorption factor of 10% should be converted to equivalent oral doses and compared to the oral NOEL.